

Fig. S1. expression of Fgf and Shh pathway genes is similar in $rx3^{-/-}$ mutants and siblings. (A-L) Sagittal (A,B; G,H) and frontal sections at the level of the forming telencephalon, optic vesicles and hypothalamus (C-F;I-L), with dorsal up. Genes analysed and genotypes are shown bottom left and top right respectively. Brackets in (A-B) highlight the extent of *fgf8* expression in the prospective telencephalon; arrows in (G-H) highlight the extent of *shh* expression in the anterior hypothalamus. All embryos are 10ss.

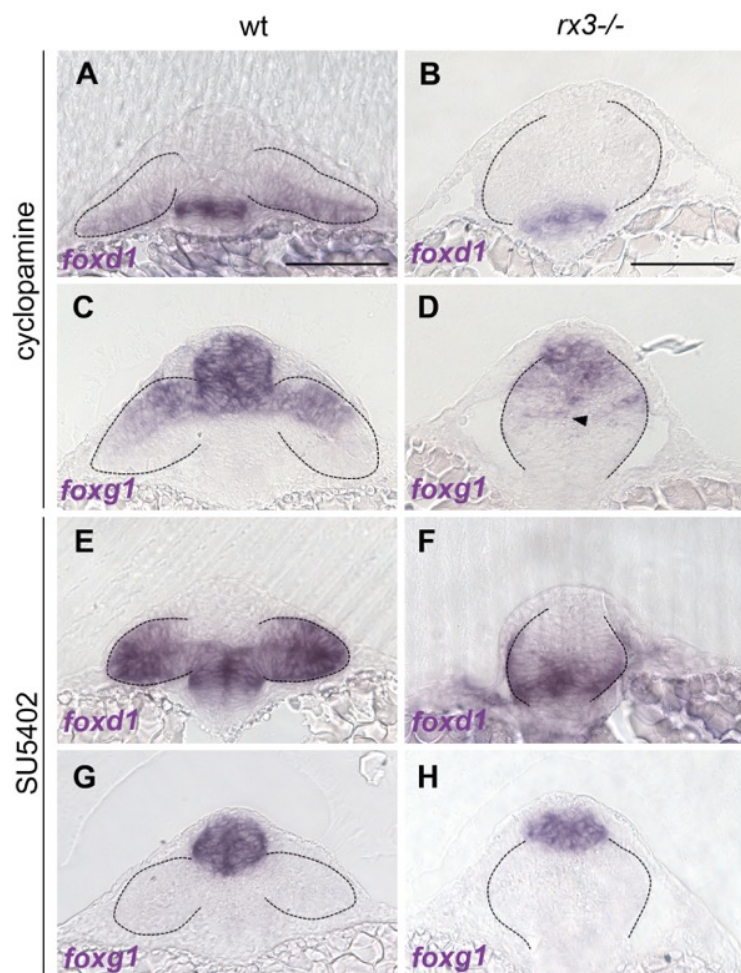


Fig. S1. abrogation of Shh or Fgf activity in *rx3* mutant embryos.

Frontal sections at the level of the forming telencephalon, optic vesicles and hypothalamus with dorsal up. Genes analysed and genotypes are shown bottom left and top respectively.

(A-D) Cyclopamine treated 10ss *rx3*^{-/-} (B,D) and sibling embryos (A,C).

(E-H) SU5402 treated 10ss *rx3*^{-/-} (F,H) and sibling embryos (E,G).

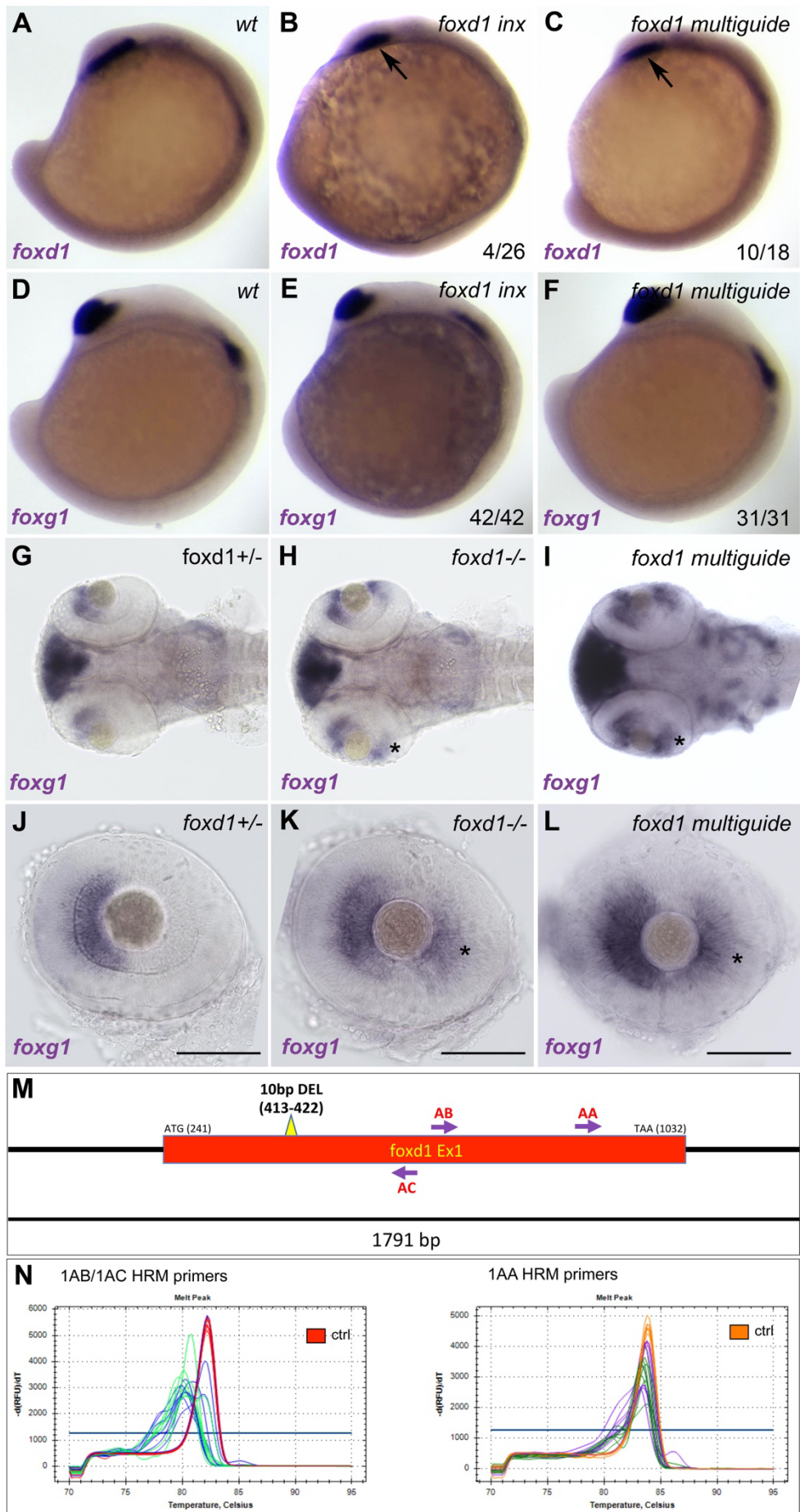


Fig. S3. *foxd1* “crispants” reproduce the *foxd1* expression expansion phenotype observed in *foxd1* mutants. (A-F) Lateral views of 12-14hpf wild type (A,D), *foxd1*^{-/-} mutants (B,E) and *foxd1* crispants (C,F) labelled with *foxd1* (A-C) or *foxd1* (D-F). Note the reduced *foxd1* domain in *foxd1* mutants (arrow, B) and crispants (arrow, C). (G-L) dorsal views of brains and eyes (G-I) and lateral views of eyes (J-L) showing *foxd1* expression in 60-72hpf wildtype (G,J), *foxd1*^{-/-} mutants (H,K) and *foxd1* crispants (I,L). Note the expansion of *foxd1* to the temporal retina (asterisk in H-I,K-L). (M) Schematic of the *foxd1* locus with the regions targeted by the CRISPR guides highlighted. (N) HRM profile in crispant embryos as compared to the wildtype profile. Two sets of primers were used to assess *indels* induced by guide 1AA (right panel) and guides 1AB/1AC (left panel). The red profile on the left panel and orange profile on the right panel correspond to wildtype controls. All genotyped embryos showed *indels* in both regions.

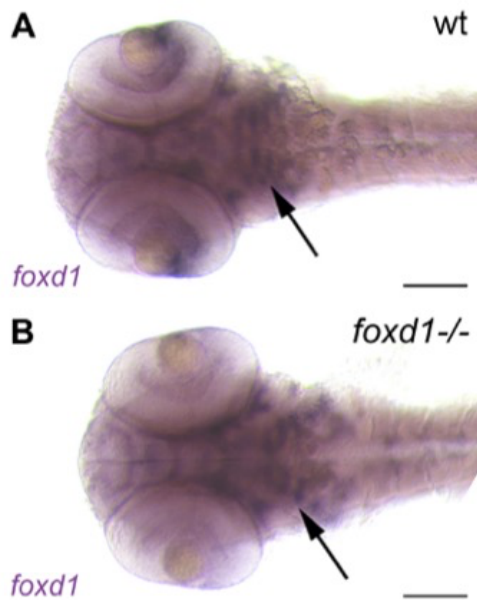


Fig. S4. loss of *foxd1* expression in the temporal retina of *foxd1* mutants is not due to nonsense-mediated RNA decay. Ventral views of heads showing *foxg1* expression in 60-72hpf wildtype (A) and *foxd1*^{-/-} mutants (B). Note *foxd1* expression in the brain and branchial arches regions (arrows) is maintained in *foxd1*^{-/-} mutants (B, compare to A), despite being absent in the retina. Anterior is to the left.

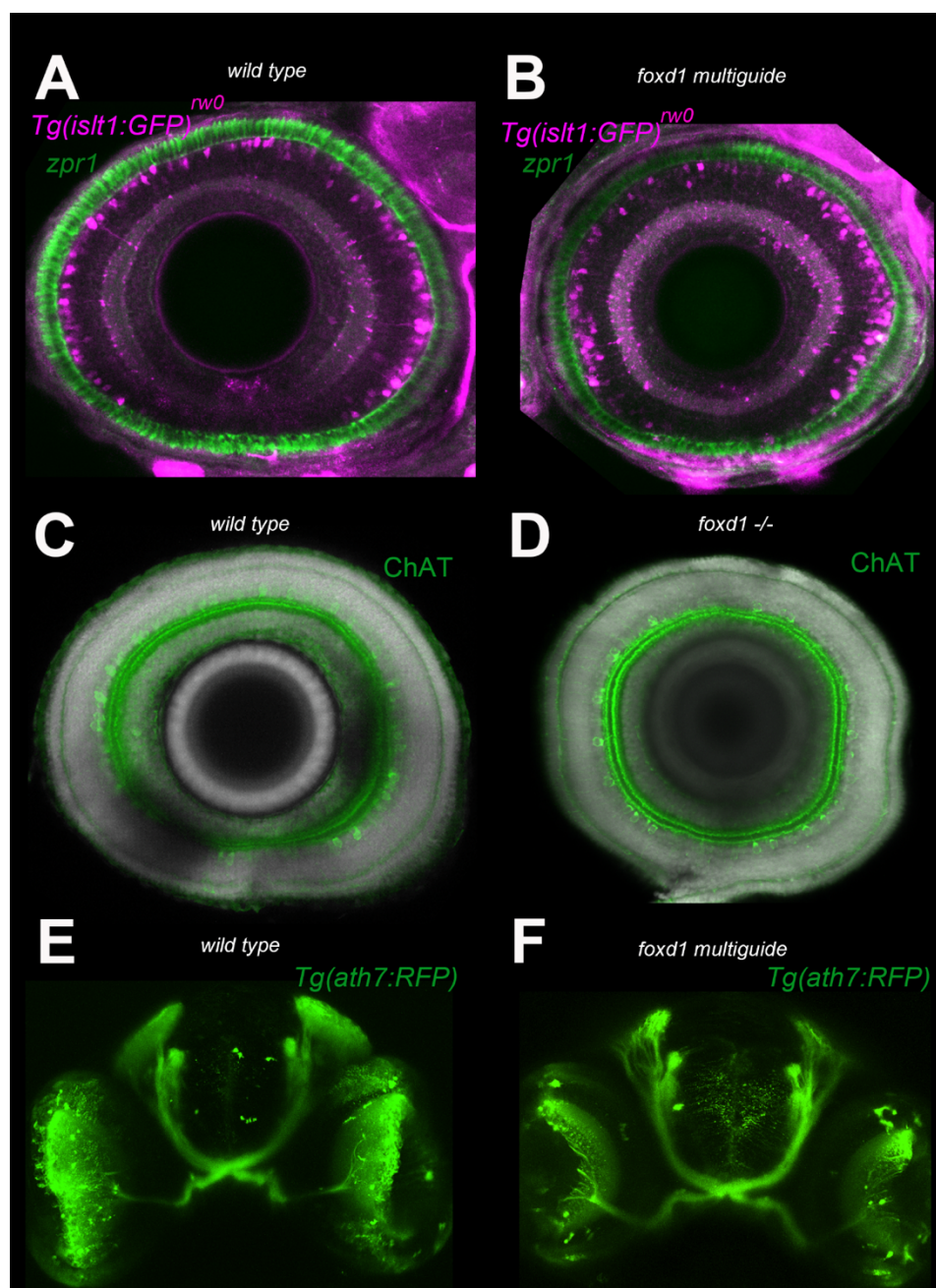


Fig. S5. retinal architecture and chiasma integrity are normal in the loss of *foxd1*. (A-B) Sagittal section across a wildtype (A) and *foxd1* crisprant (B) eye immunostained with anti-zpr1 (red and green cones, green) and anti-GFP (amacrine and retinal ganglion cells, (*Tg(islt1:GFP)*, magenta) in 8 dpf larvae. (C-D) Immunostaining with anti-Choline Acetyltransferase (ChAT, green) and DAPI (grey) in 7 dpf wild types (C) and *foxd1*^{-/-} mutants (D). (E-F) Frontal view of wild type (E) and *foxd1* crisprant (F) 4 dpf larvae highlighting RGC projections (*tg(ath7:GFP)*, green). All crisprant embryos analysed carry *indels* as confirmed by HRM analysis (not shown).

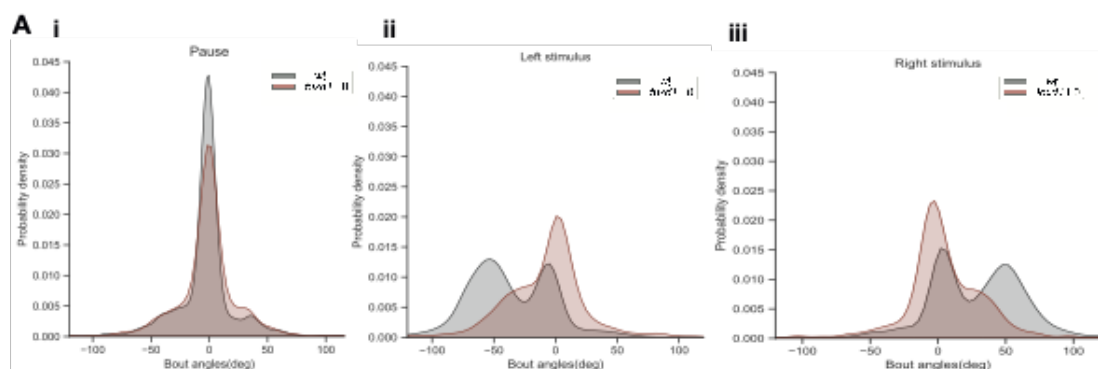
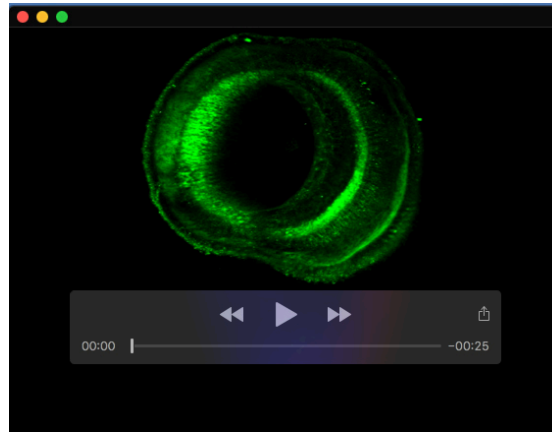


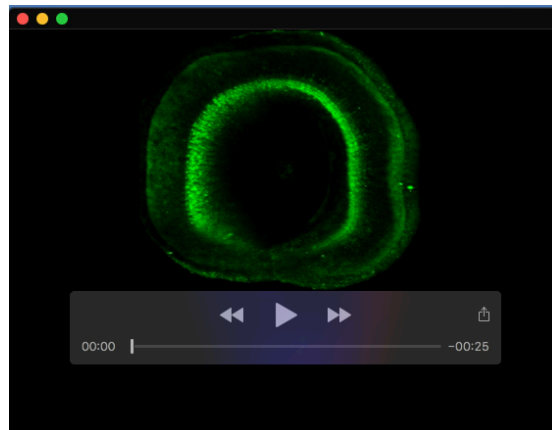
Fig. S6. range of swim bout angles is similar in wildtype and *foxd1* larvae. Probability densities of swim bout angles for wildtype and *foxd1* crispant larvae, during the pause interval (i), the leftwards (ii) and rightwards (iii) oriented whole field motion stimulus.

Table S1. Crispr guides and genotyping primers

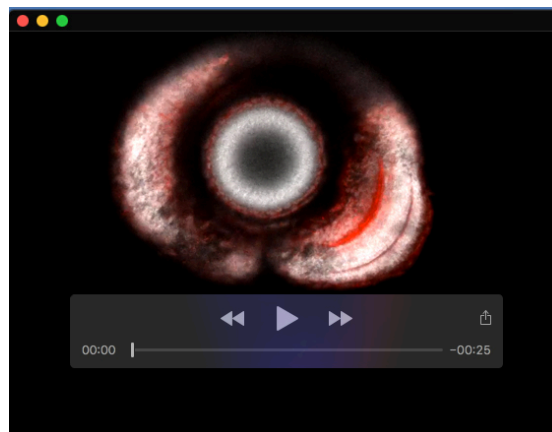
Primers used	Sequence
foxd1 original CRISPR guide	GCTTGTAGGGGTCCCGTGC
foxd1 original CRISPR-STAT-fwd	TGTAACACGACGGCCAGTTCAGATGCACGACGAGATC
foxd1 original CRISPR-STAT-rev	GTGTCTTGTCACAAATCTCGCTCAGC
foxd1 original CRISPR HRM-fwd	CAGATGCACGACGAGATCCT
foxd1 original CRISPR HRM-rev	GAAGTCACAAATCTCGCTCAGC
foxd1.1AA guide	GGCTATGGACCCTACGGTTG
foxd1.1AB guide	TCAAGATACCACGAGAGCCC
foxd1.1AC guide	CGGATAGAGTTCTGCCAAGC
foxd1-1AB/1AC HRM-fwd	GCTGAGCGAGATTTGTGACTTC
foxd1-1AB/1AC HRM-rev	CTAGCGTCCAGTAGTTGCCTTTG
foxd1-1AA HRM-fwd	GCGAGCACGGAGGTTTTCTTCC
foxd1-1AA HRM-rev	GAAAGGCGAGGAGCGCGGAATG



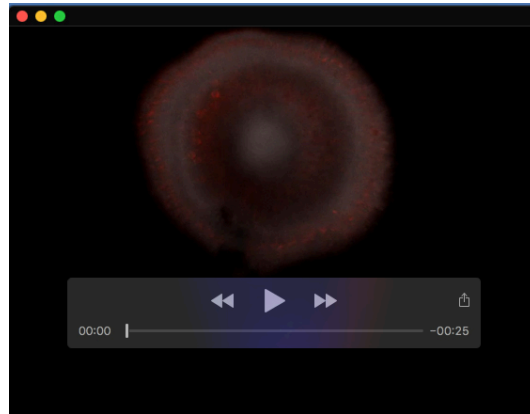
Movie 1. 3D Imaging of a 7dpf wildtype eye immunostained with anti-*PKCa* antibody (green). All movies are oriented with nasal to the left and dorsal up.



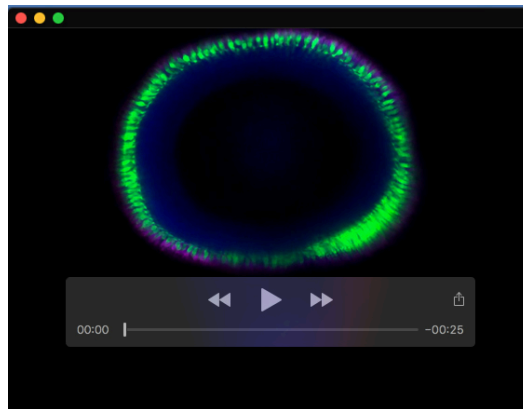
Movie 2. 3D Imaging of a 7dpf *foxd1*^{-/-} mutant eye immunostained with anti-*PKCa* antibody (green).



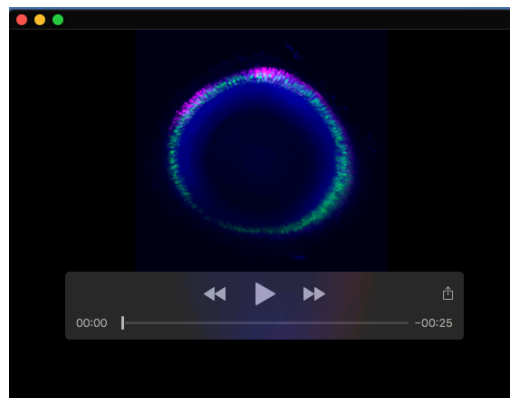
Movie 3. z-stack of a 7dpf wildtype eye immunostained with anti-ChAT (red) and counterstained with DAPI (grey).



Movie 4. z-stack of a 7dpf *foxdl*^{-/-} mutant eye immunostained with anti-ChAT (red) and counterstained with DAPI (grey).



Movie 5. z-stack of a 8dpf *Tg(opn1sw1:GFP)*, wildtype eye immunostained with anti-GFP (UV cones, green), anti-zpr1 (green and red cones, magenta) and counterstained with DAPI (blue).



Movie 6. z-stacks of a 8dpf *Tg(opn1sw1:GFP)*, *foxdl* crispant eye immunostained with anti-GFP (UV cones, green), anti-zpr1 (green and red cones, magenta) and counterstained with DAPI (blue).